Heme oxygenase-derived carbon monoxide promotes arteriolar endothelial dysfunction and contributes to salt-induced hypertension in Dahl salt-sensitive rats


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Vascular tissues express heme oxygenase (HO), which metabolizes heme to form carbon monoxide (CO). Heme-derived CO inhibits nitric oxide synthase and promotes endothelium-dependent vasconstriction. After 4 wk of high-salt diet, Dahl salt-sensitive (Dahl-S) rats display hypertension, increased vascular HO-1 expression, and attenuated vasodilator responses to ACh that can be completely restored by acute treatment with an inhibitor of HO. In this study, we examined the temporal development of HO-mediated endothelial dysfunction in isolated pressurized first-order gracilis muscle arterioles, identified the HO product responsible, and studied the blood pressure effects of HO inhibition in Dahl-S rats on a high-salt diet. Male Dahl-S rats (5–6 wk) were placed on high-salt (8% NaCl) or low-salt (0.3% NaCl) diets for 0–4 wk. Blood pressure increased gradually, and responses to an endothelium-dependent vasodilator, ACh, decreased gradually with the length of high-salt diet. Flow-induced dilation was abolished in hypertensive Dahl-S rats. Acute in vitro pretreatment with an inhibitor of HO, chromium mesoporphyrin (CrMP), restored endothelium-dependent vasodilation and abolished the differences between groups. The HO product CO prevented the restoration of endothelium-dependent dilation by CrMP. Furthermore, administration of an HO inhibitor lowered blood pressure in Dahl-S rats with salt-induced hypertension but did not do so in low-salt control rats. These results suggest that hypertension and HO-mediated endothelial dysfunction develop gradually and simultaneously in Dahl-S rats on high-salt diets. They also suggest that HO-derived CO underlies the impaired endothelial dysfunction and contributes to hypertension in Dahl-S rats on high-salt diets.

The major source of endogenous carbon monoxide formation is the enzymatic degradation of heme by heme oxygenase (40). Numerous tissues (29), including vascular endothelial and smooth muscle cells, express heme oxygenase (6, 12). The two major active isoforms of heme oxygenase (29) are the inducible heme oxygenase-1 and the constitutive heme oxygenase-2. Pathological conditions (29), such as angiotensin II-induced (17, 18), Dahl/Rapp salt-sensitive (19), or DOCA-salt hypertension (20), can increase vascular heme oxygenase-1 expression. Although carbon monoxide can relax vascular smooth muscle (10, 11, 24), it can also interfere with the vasodilator effects of the nitric oxide system (31, 41, 44) and can promote endothelium-dependent vasoconstriction (21, 36).

Dahl salt-sensitive (Dahl-S) rats are genetic models of salt-induced hypertension because they develop hypertension on a high-salt diet but remain relatively normotensive on a low-salt diet (35). Decreased endothelium-dependent vasodilation, at least in part due to decreased endothelium-derived nitric oxide function, has been repeatedly demonstrated in Dahl-S rats with established salt-induced hypertension (14, 28, 46). Numerous studies have suggested that decreased nitric oxide formation contributes to the development of salt-induced hypertension in Dahl-S rats (4, 5, 15, 30). Our previous study suggested that, in skeletal muscle arterioles isolated from Dahl-S rats after 4 wk of high-salt diet, increased heme oxygenase function contributes to endothelial nitric oxide dysfunction (19). Studies that have explored the temporal development of the dysfunction in this model are, however, scarce. In addition, there have been no studies that have evaluated whether heme oxygenase directly contributes to the hypertension that is characteristic for this model of salt-induced hypertension.

Hence, in this study, we examined the temporal development of heme oxygenase-mediated endothelial dysfunction, identified the heme oxygenase product promoting endothelial dysfunction, and studied the blood pressure effects of heme oxygenase inhibition in Dahl-S rats on high-salt diets. Toward this end, we examined endothelial function in skeletal muscle arterioles taken from Dahl-S rats after 0–4 wk of high- or low-salt diets and measured the responses to an endothelium-dependent vasodilator and to increases in luminal flow while in the presence or absence of an inhibitor of heme oxygenase. To determine the contribution of heme oxygenase activity to hypertension, we administered a heme oxygenase inhibitor or vehicle to anesthetized intact Dahl-S rats acutely instrumented with carotid arterial catheters.

METHODS

Materials. Chromium mesoporphyrin (CrMP) and zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG) were purchased from Frontier Scientific (Logan, UT); these metalloborphyrins were used in our study...
because they are potent and selective inhibitors of heme oxygenase activity (2, 42, 43). Although solubility and photostability make CrMP a preferred choice for in vitro studies, ZnDPBG is ideal for in vivo experiments because of its quick distribution. Thiobutabarbital sodium (Inactin) and ACh were obtained from Sigma Aldrich (St. Louis, MO). All other chemicals were obtained from Fisher Scientific (Huntsville, TX). CrMP stock solution (15 mmol/l) was prepared in 50 mmol/l Na2CO3 solution and diluted in modified Krebs buffer (15 mmol/l) immediately before use. ZnDPBG solution (15 mmol/l) was also prepared in 50 mmol/l Na2CO3 solution for intraperitoneal administration. ACh stock solution (10 mmol/l) was prepared in saline and diluted in modified Krebs buffer immediately before use. The composition of modified Krebs buffer was (in mmol/l) 118.5 NaCl, 4.7 KCl, 1.4 CaCl2, 1.2 KH2PO4, 1.1 MgSO4, 25.0 NaHCO3, and 11.1 dextrose.

Animals. Male inbred Dahl/Rapp salt-sensitive (Dahl-S) rats (SS/JrHsd colony, n = 96) were purchased from Harlan (Indianapolis, IN) at 4–5 wk of age. All animals were housed in a controlled environment and allowed to acclimate for 1 wk while having free access to low-salt (0.3% NaCl) synthetic rodent diet (Dyets, Bethlehem, PA) and tap water. A subset of these animals then served as a control for both diet groups (referred to as “0 wk”). Remaining animals had free access to high-salt (8.0% NaCl) or low-salt diets for an additional 1–4 wk. All animal procedures were approved by our institutional animal care and use committee.

Blood pressure measurements. On the day of the experiment, rats were weighed and anesthetized with a single dose of 100 mg/kg ip thiobutabarbital sodium (Inactin), and a carotid arterial catheter was implanted for acute determination of blood pressure and heart rate. The carotid catheter was connected to a pressure transducer (TSD 104A, Biopac Systems, Santa Barbara, CA) coupled to a polygraph system (Biopac Systems) and a personal computer. After catheter implantation, animals were used for either microvessel experiments (study 1) or intact animal experiments (study 3).

Heme oxygenase-1 protein measurements. Harvested aortic segments (study 1) were snap frozen in liquid nitrogen and stored at −70°C until analyzed. Heme oxygenase-1 protein expression was determined by Western blotting, as previously detailed (27). Briefly, tissue samples were thawed at room temperature and lysed in electrophoresis buffer [125 mM Tris-HCl (pH 6.8), 12.5% glycerol, and 2%/SDS], boiled, and sonicated. Proteins (20 μg) were separated by SDS-PAGE and electrophoretically transferred to nitrocellulose membranes. Ponceau staining was used to verify equal protein loading in all lanes. After repeated washing and completed, the membranes were blocked for 1 h in PBS containing Tween 20 (0.1%) and nonfat milk (5%). Blots were incubated with the heme oxygenase-1 antibody (1:500 dilution) for 1 h. Membranes were then incubated for 1 h with hors eradish peroxidase-conjugated goat anti-rabbit antibody (1:1,500 dilution). Blots were developed by the enhanced chemiluminescence method (Amersham), and relative protein levels were quantified by scanning densitometry (LKBI Ultrascan, XL, laser densitometer).

Study 1: time course experiments. Experiments examined the temporal development of heme oxygenase-mediated endothelial dysfunction in Dahl-S rats. Animals after 1 wk of acclimatization on low-salt diets were divided into seven time course groups. A group of 0 wk animals served as a control for both high- and low-salt diet groups. Remaining animals were used for experiments after an additional 1, 2, 3, or 4 wk of high-salt or 2 or 4 wk of low-salt diet treatments. After catheter implantation, stable blood pressure readings were obtained. Animals were then heparinized (1,000 U/kg iv), and the heart, right kidney, segments of the thoracic and abdominal aorta, and the gracilis anticus muscles were removed and placed into ice-cold modified Krebs buffer. Right kidney and heart (wet) weights were then determined. Aorta segments were used for heme oxygenase-1 protein measurements.

Segments of first-order gracilis muscle arterioles were isolated by microdissection (38). Individual arteriolar segments were cannulated at both ends with glass micropipettes in a vessel chamber (Living Systems Instrumentation, Burlington, VT). The distal micropipette was connected to a stopcock, and the proximal micropipette was connected to a reservoir whose height was adjusted to 108.8 cm to achieve 80 mmHg intraluminal pressure. The vessel chamber was continuously superfused with gassed buffer (14% O2:5% CO2:balance N2; 37°C) via a nonrecirculating system. For internal diameter measurements, the vessel chamber was mounted on the stage of an inverted microscope (Nikon TS100-F) that was fitted with a charge-coupled device video camera. The camera was connected to the video card of a personal computer equipped with video dimensioning software (ImagePro Express, Media Cybernetics). With this setup, a magnified image of the arteriolar segment was viewed on the computer screen, and the internal diameter was measured by manually adjusting the guides superimposed by the video dimensioning software. Internal diameter was recorded once each minute throughout the experiment, and arteriolar images were collected at 1 frame/s and stored as digital files for further analyses. During the 60-min stabilization period, internal diameters of arterioles decreased spontaneously in all groups. After the stabilization period, the heme oxygenase inhibitor (2, 43), 15 μmol/l CrMP, or matched vehicle was included in the superfusion buffer 20 min before the experiment. This pretreatment regime was continued throughout the remainder of the experiment. After the pretreatment period, increasing concentrations of an endothelium-dependent vasodilator, ACh (1 μmol and 1-3 μmol/l), were tested. Each concentration was tested for 5 min, and the average of the last two measurements was used to determine the magnitude of the response.

Study 2: flow response experiments. These experiments were designed to identify the role of heme oxygenase-1-derived carbon monoxide in endothelial dysfunction in hypertensive Dahl-S rats by use of an alternate stimulus for endothelial nitric oxide production independent of muscarinic receptor activation. After 1 wk of acclimatization, rats had free access to high- or low-salt diets for an additional 4 wk. On the day of the experiments, animals were anesthetized with a single injection of Inactin (100 mg/kg ip) and heparinized (1,000 U/kg iv), and gracilis anticus muscles were removed. Segments of first-order gracilis muscle arterioles were isolated by microdissection (38). Individual arteriolar segments were cannulated at both ends with glass micropipettes in a vessel chamber (Living Systems Instrumentation). Both the proximal and distal micropipettes were connected to pressure servo controllers (Living Systems Instrumentation) and an in-line microflowmeter (Living Systems Instrumentation). The heme oxygenase inhibitor (2, 43), 15 μmol/l CrMP, or vehicle was included in the luminal perfusion buffer. During a 60-min stabilization period, both proximal and distal pressures were adjusted to 80 mmHg with no luminal flow. During the experiments, proximal and distal pressures were adjusted equally in opposite directions to maintain the midline pressure at 80 mmHg and to establish graded levels of luminal flow (0–50 μl/min in 5 μl/min increments). Each flow was tested for 5 min, internal diameter was recorded every minute, and the average of the last two measurements was used to determine the response. The vessel chamber was continuously superfused with gassed buffer (14% O2:5% CO2:balance N2; 37°C) via a nonrecirculating system. Internal diameter was measured as described in study 1.

To identify the role of the heme oxygenase product carbon monoxide in heme oxygenase-mediated endothelial dysfunction, additional arterioles from high-salt animals were simultaneously exposed to 15 μmol/l CrMP, a heme oxygenase inhibitor, and 100 μmol/l carbon monoxide via the luminal perfusion buffer. Flow-induced responses were then determined.

Study 3: intact animal experiments. Experiments were performed to determine blood pressure effects of systemic heme oxygenase inhibition in Inactin-anesthetized Dahl-S rats. After 1 wk of acclimatization, rats had free access to high- or low-salt diets for an additional 4 wk. On the day of the experiments, animals were anesthetized with 100
mg/kg ip inactin and implanted with indwelling carotid arterial catheters, and blood pressure and heart rate were measured as described above. For this intact animal series, ZnDPBG was selected as the preferred heme oxygenase inhibitor because it has been documented to be quickly distributed in vivo (24, 43). After a 15-min stabilization period, arterial pressures and heart rates were measured for 15 min before and 30 min after bolus intraperitoneal administration of 15 μmol/kg ZnDPBG, a heme oxygenase inhibitor, or vehicle (0.5 ml of 50 mmol/l Na2CO3).

Statistics. All data are expressed as means ± SE. Nonlinear curve fitting was performed on individual ACh concentration-response curves for determination of EC50 with GraphPad Prism 3.03 computer software. A statistical package (Sigmastat 3.0) was used to perform one-way ANOVA to analyze EC50 values and maximum responses for vascular experiments and mean arterial pressures, heart rates, and organ weights for the animals. As necessary, orthogonal contrasts were performed (Systat 10.2) as post hoc tests, to compare each group with the control (0 wk) group (37). P < 0.05 was considered statistically significant.

RESULTS

Study 1: time course experiments. In Dahl-S rats, mean arterial pressure increased gradually (P < 0.05) with the length of high-salt diet and peaked after 3 wk with no changes in heart rate (Tables 1 and 2). Kidney weights and kidney-to-body weight ratios also increased (P < 0.05) gradually with the length of high-salt diet (Tables 1 and 2). Heart weights increased, and heart-to-body weight ratios also increased with the length of high-salt diet (P < 0.05) (Tables 1 and 2). Mean arterial pressures, heart rates, kidney and heart weights, and heart-to-body weight ratios did not change significantly in Dahl-S rats on low-salt diets (Table 1). Kidney-to-body weight ratios actually decreased with the length of low-salt diets (P < 0.05) (Table 1).

In aorta segments, heme oxygenase-1 protein content was increased after 4 wk (P < 0.05) but not after 2 wk of high-salt diet compared with 0 wk controls (Fig. 1).

During the stabilization period, the internal diameter of isolated skeletal muscle arterioles decreased spontaneously in all groups (0 wk: 182 ± 4 to 129 ± 5 μm, n = 7; 1 wk high-salt: 170 ± 4 to 124 ± 6 μm, n = 14; 2 wk high-salt: 186 ± 3 to 144 ± 3 μm, n = 16; 3 wk high-salt: 189 ± 3 to 156 ± 8 μm, n = 10; 4 wk high-salt: 186 ± 5 to 162 ± 6 μm, n = 11; 2 wk low-salt: 200 ± 4 to 150 ± 6 μm, n = 7; 4 wk low-salt: 208 ± 3 to 157 ± 7 μm, n = 10). Pretreatment with the heme oxygenase inhibitor CrMP (15 μmol/l) restored ACh-induced vasodilator re-

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Values are means ± SE. Dahl-S, Dahl-salt-sensitive rats; 0 week, control Dahl-S rats (n = 4); 2 weeks, Dahl-S rats after 2 wk of low-salt diet (n = 4); 4 wk, Dahl-S rats after 4 wk of low-salt diet (n = 9); MAP, mean arterial pressure; HR, heart rate. *P < 0.05 compared with 0 wk group.
Fig. 2. Concentration-dependent effects of an endothelium-dependent vasodilator, ACh, on changes in internal diameter of first-order gracilis muscle arterioles isolated from Dahl-S rats after 0 wk [vehicle, n = 4; chromium mesoporphyrin (CrMP), n = 3], 1 wk (vehicle, n = 6; CrMP, n = 8), 2 wk (vehicle and CrMP, n = 8 each), 3 wk (vehicle and CrMP, n = 5 each), or 4 wk (vehicle, n = 7; CrMP, n = 4) of high-salt diets. Arterioles were acutely pretreated in vitro with vehicle (A) or with an inhibitor of heme oxygenase, 15 μmol/l CrMP (B), for 20 min before experiments. Data are expressed as means ± SE. Maximum responses of vehicle- and CrMP-treated arterioles are significantly different from 0 wk after 2 wk of high-salt diet (P < 0.05). Other maximal responses and EC50 values are not different (P = not significant) from the respective 0 wk controls.

Fig. 3. Concentration-dependent effects of an endothelium-dependent vasodilator, ACh, on changes in internal diameter of first-order gracilis muscle arterioles isolated from Dahl-S rats after 0 wk (vehicle, n = 4; CrMP, n = 3), 2 wk (vehicle, n = 4; CrMP, n = 3), or 4 wk (vehicle, n = 6; CrMP n = 4) of low-salt diets. Arterioles were acutely pretreated in vitro with vehicle (A) or with an inhibitor of heme oxygenase, 15 μmol/l CrMP (B), for 20 min before experiments. Data are expressed as means ± SE. Vehicle-treated arterioles’ maximum responses are significantly different from 0 wk after 4 wk of low-salt diet (P < 0.05). Other maximal responses and EC50 values are not different (P = not significant) from the respective 0 wk controls.

In Dahl-S rats after 4 wk of high-salt diets, acute administration of the heme oxygenase inhibitor CrMP (15 μmol/l) and the heme oxygenase product carbon monoxide (mean system concentration of 100 μmol/l) prevented the restoration of flow-induced dilation in high-salt arterioles (Δmax = 0 ± 1 μm, n = 5) (Fig. 4B).

Study 3: intact animal experiments. In Dahl-S rats after 4 wk of high-salt diets, acute administration of the heme oxygenase inhibitor ZnDPBG (15 μmol/kg ip) lowered mean arterial pressure without affecting heart rate [ZnDPBG: 198 ± 5 to 161 ± 5 mmHg (n = 5) vs. vehicle: 193 ± 8 to 186 ± 9 mmHg (n = 6); P < 0.05] (Fig. 5A). In age-matched Dahl-S rats on low-salt diets, the heme oxygenase inhibitor did not affect blood pressure [ZnDPBG: 142 ± 5 to 134 ± 5 mmHg (n = 8) vs. vehicle: 131 ± 6 to 122 ± 2 mmHg (n = 8)] (Fig. 5B).
DISCUSSION

In this study, we found that development of hypertension in Dahl-S rats is accompanied by a gradual impairment in ACh-dependent vasodilator responses. We also found evidence that flow-induced dilation, which is also mediated by nitric oxide, can be absent in Dahl-S rats with salt-induced hypertension. Acute in vitro pretreatment with an inhibitor of heme oxygenase restored ACh- and flow-induced dilation and abolished the differences between groups. Furthermore, the heme oxygenase product carbon monoxide prevented the restoration of endothelium-dependent dilation by the heme oxygenase inhibitor. Moreover, acute administration of a heme oxygenase inhibitor lowered blood pressure in Dahl-S rats with salt-induced hypertension but not in low-salt controls.

Dahl-S rats are genetic models of salt-induced hypertension (35). In this study, we found that, in Dahl-S rats, mean arterial pressure increased gradually with the length of high-salt diet. In contrast, low-salt diet did not increase blood pressure even after 4 wk. This salt-induced hypertension was accompanied by a gradual increase in kidney and heart weights in Dahl-S rats, potentially indicative of gradual development of renal (7, 13) and cardiac (8, 14) damage. Our findings suggest that hypertension and heme oxygenase-mediated endothelial dysfunction develop gradually and simultaneously in Dahl-S rats on high-salt diets.

Carbon monoxide is formed in vivo mainly via the enzymatic breakdown of heme by heme oxygenase (40). Pathological conditions, such as angiotensin II-induced (17, 18) or DOCA-salt (20) hypertension can increase vascular heme oxygenase-1 expression. We previously showed that vascular heme oxygenase-1 expression was increased in Dahl-S rats after 4 wk of high-salt diet (19). In the present study, we found that aortic heme oxygenase-1 protein content is significantly increased after 4 wk but not after 2 wk of high-salt diet. These findings suggest that vascular heme oxygenase-1 induction may be temporally linked with the development of salt-induced hypertension in Dahl-S rats.

Fig. 4. Flow-induced changes in internal diameter of first-order gracilis muscle arterioles isolated from Dahl-S rats after 4 wk of low-salt (vehicle, n = 6; CrMP n = 7) or high-salt diets (vehicle and CrMP, n = 5 each). Arterioles were acutely pretreated in vitro with vehicle (A), with an inhibitor of heme oxygenase, 15 μmol/l CrMP (B), or simultaneously with CrMP and a heme oxygenase product, 100 μmol/l carbon monoxide (■; n = 5 high-salt), for 20 min before experiments. Data are expressed as means ± SE. *P < 0.05 relative to low-salt group. †P < 0.05 relative to high-salt CrMP-pretreated arterioles.

Fig. 5. Heart rate (HR; top) and mean arterial pressure (MAP; bottom) of anesthetized Dahl-S rats after 4 wk of high-salt (A) or low-salt (B) diets before and after intraperitoneal administration of vehicle (high-salt, n = 6; low-salt, n = 8) or an inhibitor of heme oxygenase, 15 μmol/kg zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG; high-salt, n = 5; low-salt, n = 8). Data are expressed as means ± SE. *P < 0.05 relative to control period.
Heme oxygenase-derived carbon monoxide interferes with the vasodilatory effects of the nitric oxide system (31, 41, 44) and can promote endothelium-dependent vasoconstriction (21, 36). Induction of heme oxygenase-1 has been shown to attenuate muscarinic agonist-induced nitric oxide release (41) and vasorelaxation (25) in isolated arteries. We previously found that enhanced vascular heme oxygenase function may contribute to arteriolar endothelial dysfunction in DOCA-salt hypertensive rats (20) and in Dahl-S rats after 4 wk of high-salt diet (19). Decreased endothelium-dependent vasodilation has been repeatedly demonstrated in Dahl-S rats with established salt-induced hypertension (14, 28, 46), but studies on the temporal development of endothelial dysfunction in this model are scarce. In the present study, we found that, in skeletal muscle arterioles isolated from Dahl-S rats, responses to an endothelium-dependent vasodilator, ACh, decreased gradually with the length of high-salt diet. Acute in vitro pretreatment with an inhibitor of heme oxygenase, CrMP, restored ACh-induced dilation to the same level in all groups. In contrast, low-salt diet promoted only a moderate decrease in ACh-induced vasodilation after 4 wk that was also restored by CrMP, an inhibitor of heme oxygenase. These data suggest that heme oxygenase-mediated endothelial dysfunction develops gradually in Dahl-S rats on high-salt diets. We found no significant increase in aortic heme oxygenase-1 protein content after 2 wk of high-salt diet, yet endothelial function was significantly, although moderately, impaired. After 4 wk of high-salt diet, aortic heme oxygenase-1 protein content was significantly elevated, and endothelial dysfunction was further impaired. These data suggest that vascular heme oxygenase-1 induction contributes to the development of endothelial dysfunction in Dahl-S rats on high-salt diets. The possibility exists that our Western blotting technique is not sensitive enough to show slight increases in heme oxygenase-1 protein content after 2 wk of high-salt diet or that vascular heme oxygenase activity is increased via other means, perhaps due to increased substrate availability (24, 33).

Although muscarinic agonists are widely used to assess endothelial function (25, 28, 46), high-salt diet has been shown to interfere with muscarinic receptor signaling proximal to nitric oxide synthase in non-salt-sensitive animals (39). An alternative means for assessing endothelial function, which circumvents this potential mechanism, is to generate nitric oxide in response to increased shear forces along the vascular endothelium (34). We previously reported (23) that in skeletal muscle arterioles isolated from normotensive male Sprague-Dawley rats flow-induced dilation is enhanced by an inhibitor of heme oxygenase. In contrast, flow-induced dilation is abolished by a heme precursor in a manner that could be fully prevented and reversed by the heme oxygenase inhibitor. These results suggest that flow-induced dilation, even under physiological conditions, is attenuated by a heme oxygenase product. Although the heme oxygenase product biliverdin has no effect on flow-induced dilation, another heme oxygenase product, carbon monoxide, does abolish flow-induced dilation. Exogenous carbon monoxide also abolishes flow-induced dilation in vessels simultaneously treated with the heme oxygenase inhibitor. Collectively, these results suggest that heme-derived carbon monoxide can attenuate flow-induced dilation.

In the present study, we found that flow-induced dilation was abolished in Dahl-S rats after 4 wk of high-salt diets compared with low-salt controls. Acute in vitro treatment with the heme oxygenase inhibitor CrMP restored flow-induced dilation in high-salt arterioles and abolished the differences between high-salt and low-salt groups. We further found that simultaneous treatment with the heme oxygenase product carbon monoxide prevented the restoration of flow-induced dilation by the heme oxygenase inhibitor. These data suggest that enhanced vascular heme oxygenase-derived carbon monoxide formation may contribute to diminished shear force-mediated endothelial function. Furthermore, they show that the impairment in nitric oxide function is independent of muscarinic receptor signaling mechanisms that are proximal to nitric oxide synthase.

Another heme oxygenase product, iron, has been suggested to promote the generation of reactive oxygen species (32); thus it could potentially attenuate endothelium-dependent vasodilation by inactivating nitric oxide. We recently released a preliminary report that the membrane-permeable superoxide dismutase mimetic Tempol (4-hydroxy-2,2,6,6-tetramethyl-piperidine 1-oxyl) (100 μmol/l pretreatment for 20 min) does not acutely enhance ACh-induced vasodilation in Dahl salt hypertensive arterioles (22). Accordingly, it suggests that reactive oxygen species are not likely to be acute contributors to endothelial dysfunction in Dahl-S rats.

Heme oxygenase-1 overexpression has been suggested to interfere with soluble guanylate cyclase activity (16). However, our group (19) has previously demonstrated that vasodilator responses to a nitric oxide donor are not impaired in skeletal muscle arterioles isolated from Dahl salt-hypertensive rats. These data argue against heme oxygenase affecting nitric oxide signaling or bioavailability as a major mechanism of endothelial dysfunction in hypertensive Dahl-S rats.

It has also been suggested that salt can enhance the expression of inducible nitric oxide synthase in Dahl salt-resistant rats, but this effect is impaired in the Dahl-S strain (30). Accordingly, one might speculate that CrMP-induced improvements in endothelial function may arise by permitting the enhanced expression of inducible nitric oxide synthase. However, it seems unlikely that the heme oxygenase inhibitor could appreciably increase inducible nitric oxide synthase expression in just 20 min. Because it has been shown that carbon monoxide can directly bind to and inhibit activity in all three isoforms of nitric oxide synthase (1, 41, 44), it is most likely that the endothelial dysfunction seen in hypertensive Dahl-S rats arises from the inhibition of nitric oxide production by carbon monoxide.

Because skeletal muscle arterioles represent a substantial portion of total peripheral resistance, they are major determinants of blood pressure. Our present study found that skeletal muscle arteriolar endothelial dysfunction and hypertension develops in parallel in Dahl-S rats on high-salt diets. To examine whether heme oxygenase function contributes to hypertension, we examined the blood pressure effects of heme oxygenase inhibition in intact Dahl-S rats after 4 wk of high-salt diets. We found that administration of a heme oxygenase inhibitor acutely lowered mean arterial pressure in high-salt Dahl-S rats but not in low-salt animals.
These data suggest that heme oxygenase-mediated endothelial dysfunction may contribute to hypertension in Dahl-S rats on high-salt diets.

In summary, we found that in Dahl-S rats blood pressure increased gradually and endothelium-dependent vasodilator responses decreased gradually with the length of high-salt diet. We also found that shear force-mediated flow-induced dilation was abolished in Dahl-S rats with salt-induced hypertension. Acute in vitro pretreatment with an inhibitor of heme oxygenase restored ACh- and flow-induced dilation and abolished the differences between groups. Furthermore, the heme oxygenase product carbon monoxide prevented the restoration of endothelium-dependent dilation by the heme oxygenase inhibitor. Moreover, acute administration of a heme oxygenase inhibitor lowered blood pressure in Dahl-S rats with salt-induced hypertension but not in low-salt controls. These studies suggest that hypertension and heme oxygenase-mediated endothelial dysfunction develop gradually and simultaneously in Dahl-S rats on high-salt diets. Heme oxygenase-derived carbon monoxide also promotes impairment of physiological shear force-mediated vasodilator responses. Furthermore, heme oxygenase-derived carbon monoxide-mediated endothelial dysfunction contributes to hypertension in Dahl-S rats on high-salt diets.

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